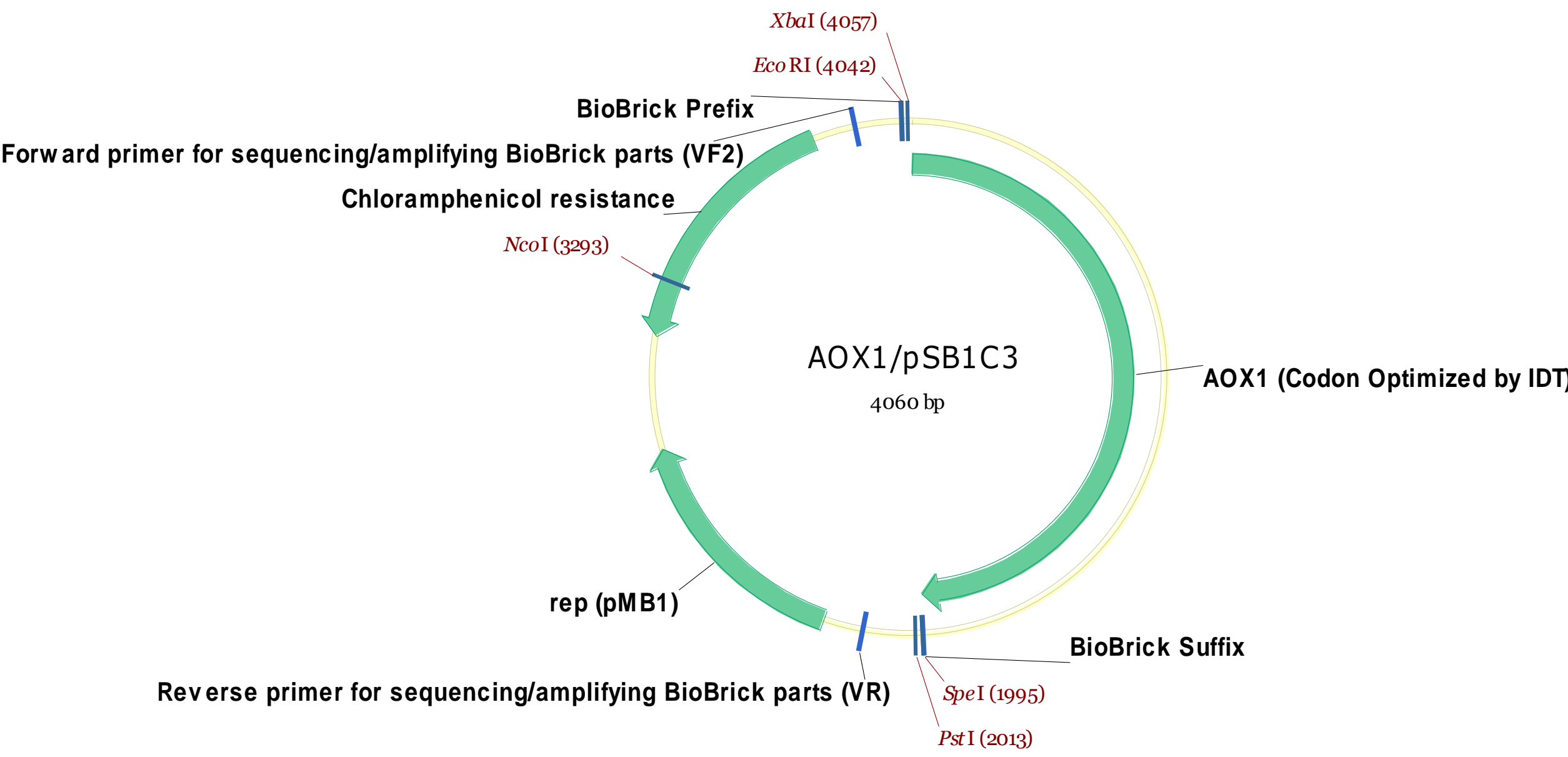


AOX1/pSB1C3



Strategy I → Failed with gBlock

- Vector: pSB1C3 (2070bp) → XbaI + PstI
- Insert: AOX1 (1992 bp) → XbaI + PstI
- PCR with the template of gBlock DNA fragment from IDT

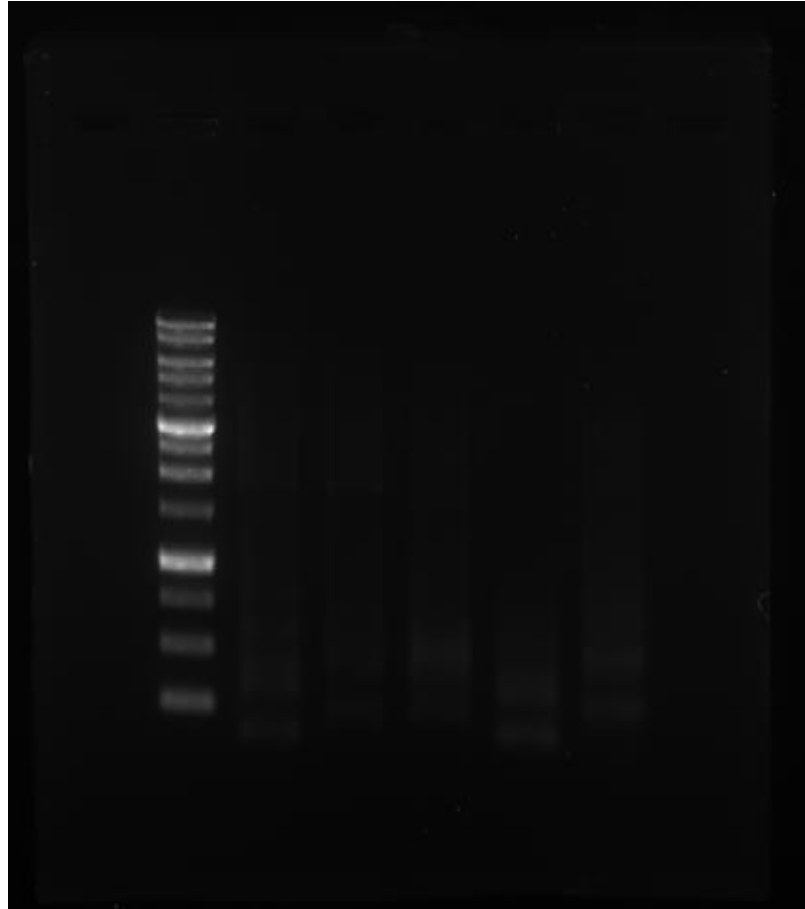
gBlocks® Gene Fragments Entry – AOX1

ATGGCTATTCCGGAAGAGTTCGATATCTTAGTGTTGGGTGGAGGTTGAGCGGGTCGTGTATTGCCGGTCGCTTG
GCCAATCTGGACCACAGCTTAAAAGTAGGTCTTATTGAAGCAGGTGAAAATAACCTGAACAACCCGTGGGTATAC
TTACCCGGAATTTACCCACGTAATATGAACTTGACAGTAAGACGGCATCGTTCTATACTAGCAATCCTTCTCCACA
TCTGAACGGTCGCCGTGCCATTGTGCCATGCGCTAACGTGCTTGGCGGTGGGTCAAGTATTAACCTTTATGATGTAT
ACGCGTGGTAGTGCTTCAGACTACGATGACTTTCAAGCCGAAGGTTGGAAAACGAAAGACCTGCTTCCTTTAATGA
AGAAAACCGAAACCTACCAGCGTGCGTGTAACAATCCTGACATCCACGGATTCTGAAGGACCAATCAAAGTGAGTT
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TCTGGAAGACCTGGTGACCGCTCACGGTGCTGAACACTGGTTAAAGTGGATCAATCGCGATACGGGTCGTCGCTC
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CCTCGCACAAAATCTATCGCGCACGCAAACAAATTGTCTTATCGTGTGGGACGATTTCTCTCCATTAGTCCTTCAG
CGCTCGGGTTTCGGGGACCCGATTAAATTGCGTGCGGCTGGTGTTAAGCCCTTAGTTAATCTTCTGGAGTTGGCC
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GCGGTGATGCCGAAATCCAGAAGCGTGTATTTGATCAGTGGTATGCAAACGGAACGGGTCCCCTTGCAACAAACG
GTATCGAGGCCGGAGTGAAGATTCGTCCCACTCCTGAAGAGTTAAGCCAGATGGACGAGAGCTTCCAGGAAGGC
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ATCCATTATTTCCCTATTCGTCTGGAGGCCCGTGCATTGGAAATGGATCTGGAGACGTCCAACGCGTATGGAGGGCC
CTTAACTTGAGCGCTGGCCTGGCTCATGGCTCCTGGACGCAACCTCTGAAGAAGCCCACTGCAAAGAACGAGGG
GCATGTAACCTCAAATCAGGTAGAACTTCACCCGGACATCGAATATGATGAGGAAGATGATAAAGCCATCGAGAA
CTATATCCGTGAACATAACGAGACCACCTGGCATTGCTTAGGCACTTGCAAGTATTGGGCCGCGTGAAGGCAGTAA
AATCGTGAAATGGGGAGGGGTTTTGGATCACCGCTCCAACGTCTACGGCGTTAAAGGGCTGAAAGTCGGGGATTT
ATCTGTATGTCCTGACAACGTGCGCTGTAACACCTACACAACGGCATTGTTGATTGGAGAGAAAACGGCTACACTG
GTTGGGGAAGACCTTGGTTATTCTGGGGAGGCGTTGGATATGACGGTCCCACAATTTAAGTTAGGAACATATGAA
AAGACCGGCCTTGCGCGCTTTTGATACTA

Primer Design

- AOX1-XbaI-F:
 - 5'- GGCGAGTCTAG ATGGCTATTCCGGAAGAGTT -3'
- AOX1-PstI-R:
 - 5'- AAAATTCTGCAGCGGCCGCTAC TAGTA TCAAAAGCGCGCAA -
3'

PCR for AOX1, AOX2, GOX with KOD-plus

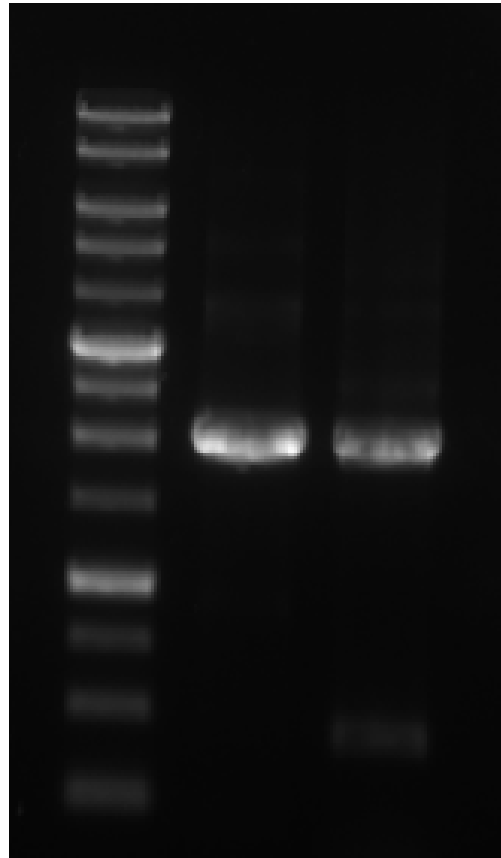


- Lane:
 1. (A1E) AOX1 with AOX1-EcoRI-F / AOX1-HindIII-R
 2. (A2E) AOX2 with AOX2-EcoRI-F / AOX2-HindIII-R
 3. (GE) GOX with GOX-EcoRI-F / GOX-HindIII-R
 4. (A1S) AOX1 with AOX1-XbaI-F / AOX1-PstI-R
 5. (A2S) AOX2 with AOX2-XbaI-F / AOX2-PstI-R
- Cloning: E for pET-29b; S for pSB1C3

Strategy II → Success

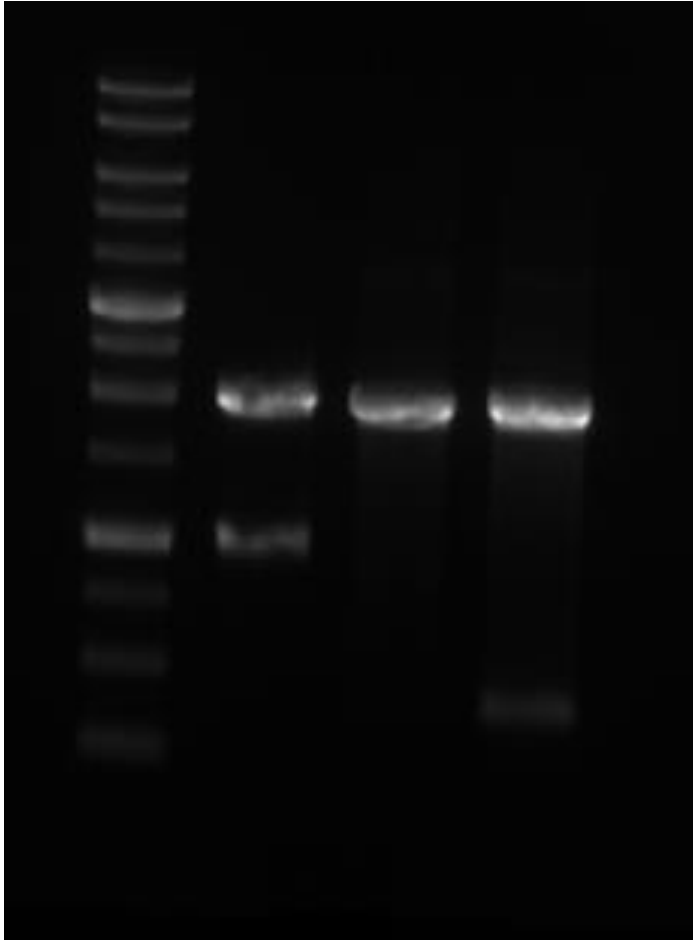
- Vector: pSB1C3 (2070bp) → XbaI + PstI
- Insert: AOX1 (1992 bp) → XbaI + PstI
- PCR with the template of AOX1/pET-29b #1-11
 - Primer: AOX1-XbaI-F & AOX1-PstI-R
 - Annealing T_m: 58 degree C because the reverse primer overlaps template with 11 bp ← No problem I used 60 degree C

PCR for AOX1, AOX2 with KOD-plus



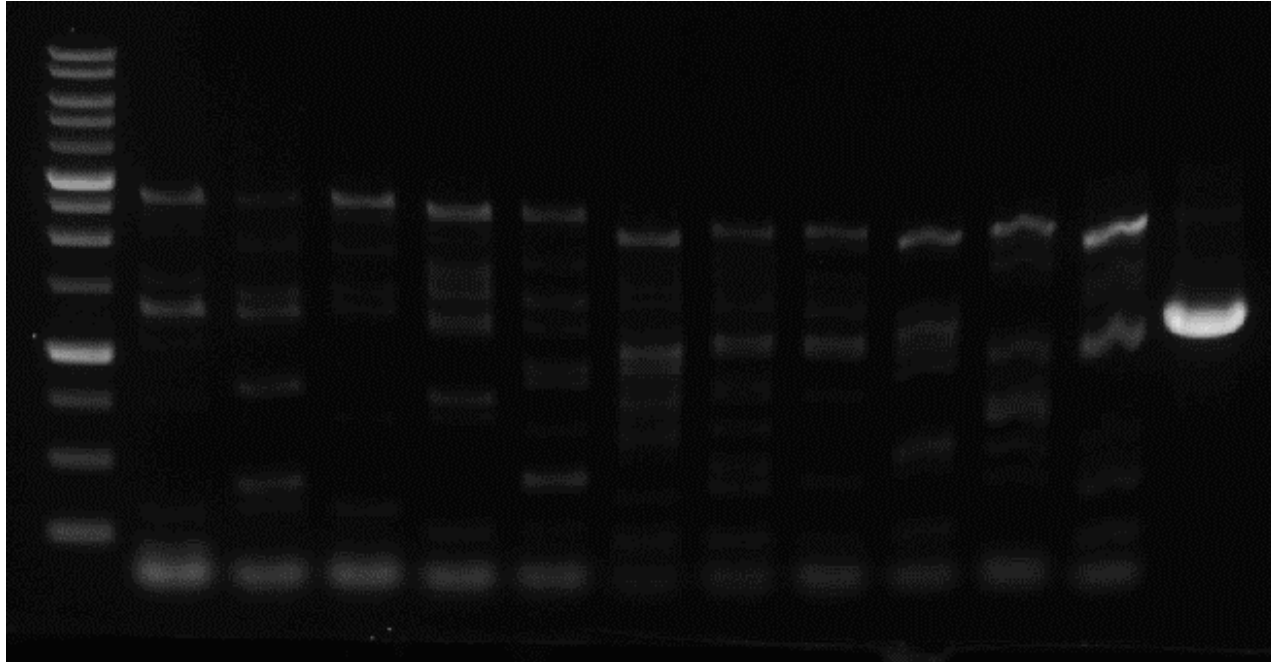
- Date: 2016.08.10
- Lane:
 1. XbaI-AOX1-PstI
 2. XbaI-AOX2-PstI

Ligation: AOX1 + pSB1C3, AOX2 + pSB1C3



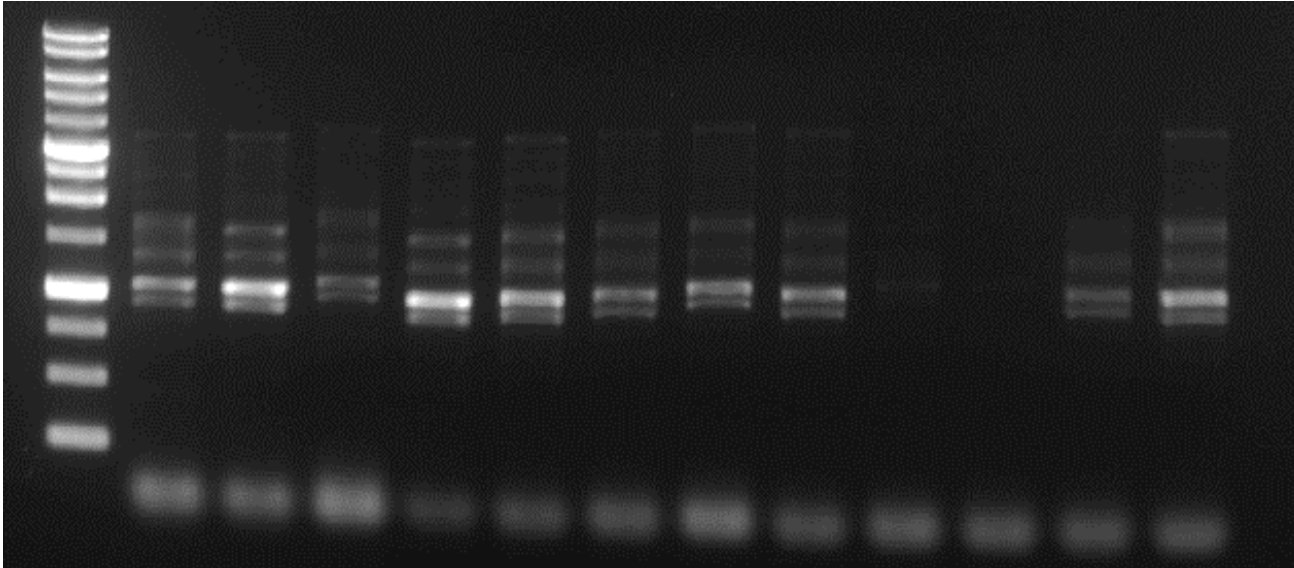
- Date: 2016.08.11
- Lane:
 1. pSB1C3: XbaI + PstI
 2. AOX1: XbaI + PstI
 3. AOX2: XbaI + PstI

Colony PCR



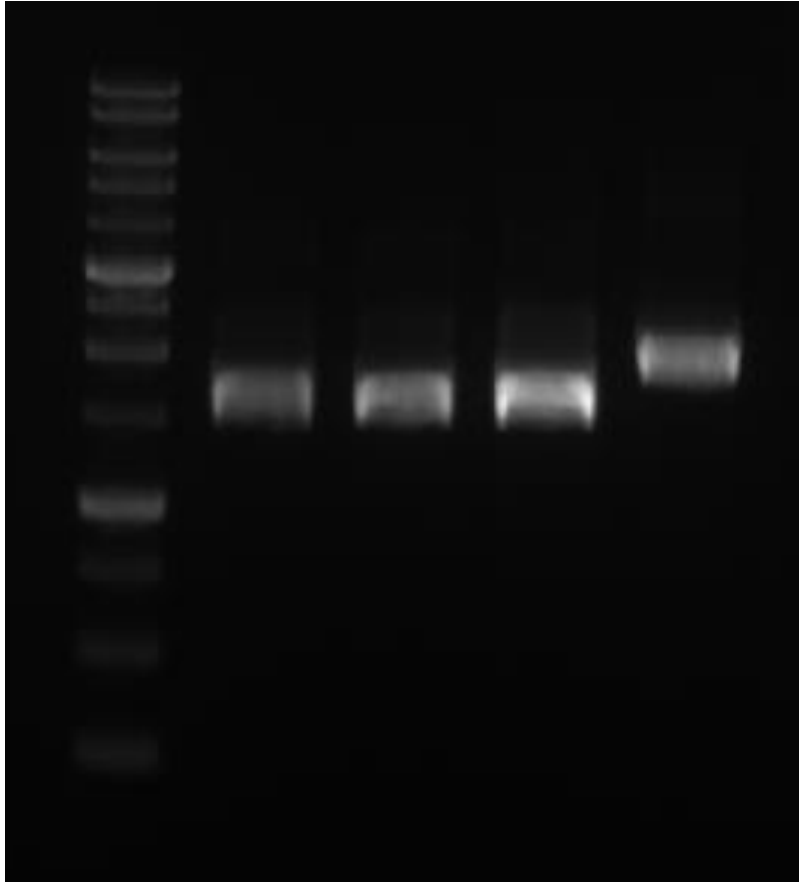
- Date: 2016.08.11
- Clone: AOX1/pSB1C3
- Primer:
 1. AOX1-Xbal-F
 2. VR
- Lane: #1-1 ~ #1-12

Colony PCR II



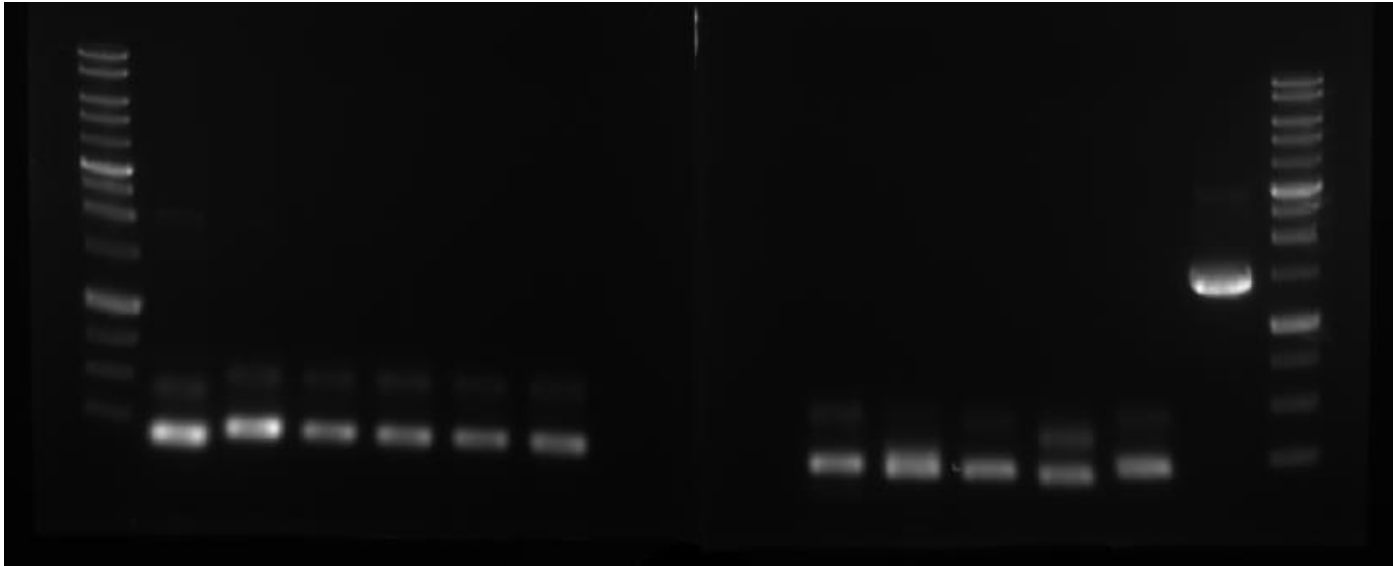
- Date: 2016.08.15
- Clone: AOX1/pSB1C3
- Primer:
 1. VF2
 2. AOX1-PstI-R
- Lane: #1-1 ~ #1-12
- #1-2, 3, 4 → Plasmid extraction → RE check

RE check



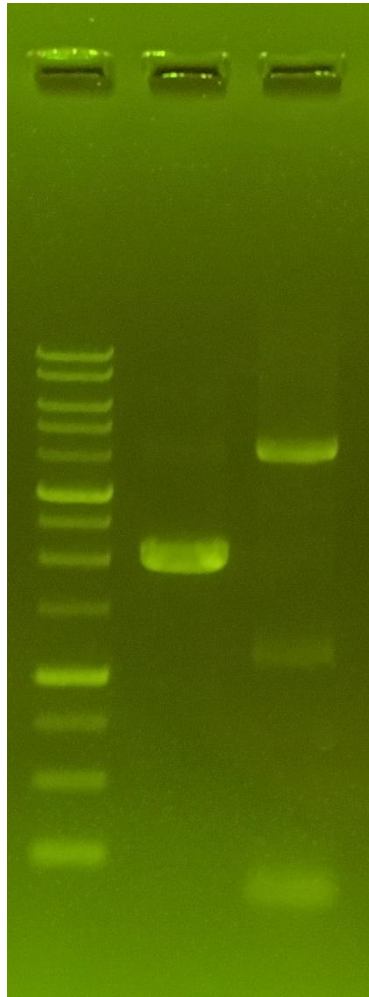
- Date: 2016.08.16
- Clone: AOX1/pSB1C3
- RE: EcoRI + PstI
- Lane:
 1. #1-2
 2. #1-3
 3. #1-4
 4. #2-7 (AOX2/pSB1C3)
- Result: **failed**

Colony PCR III



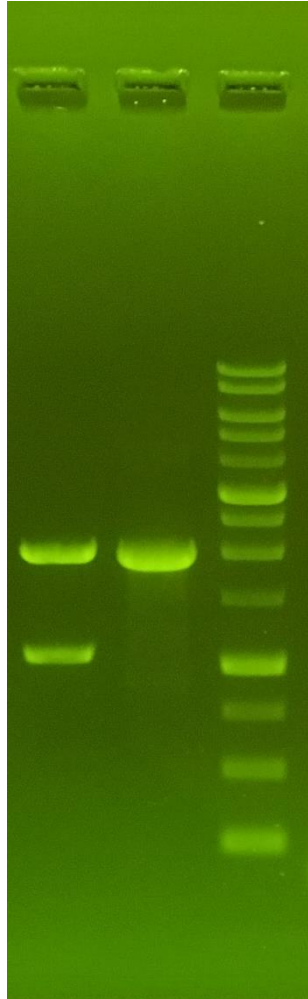
- Date: 2016.08.16
- Clone: AOX1/pSB1C3
- Primer:
 1. VF2
 2. VR
- Lane: #1-1 ~ #1-12

PCR II for AOX1 with KOD-plus



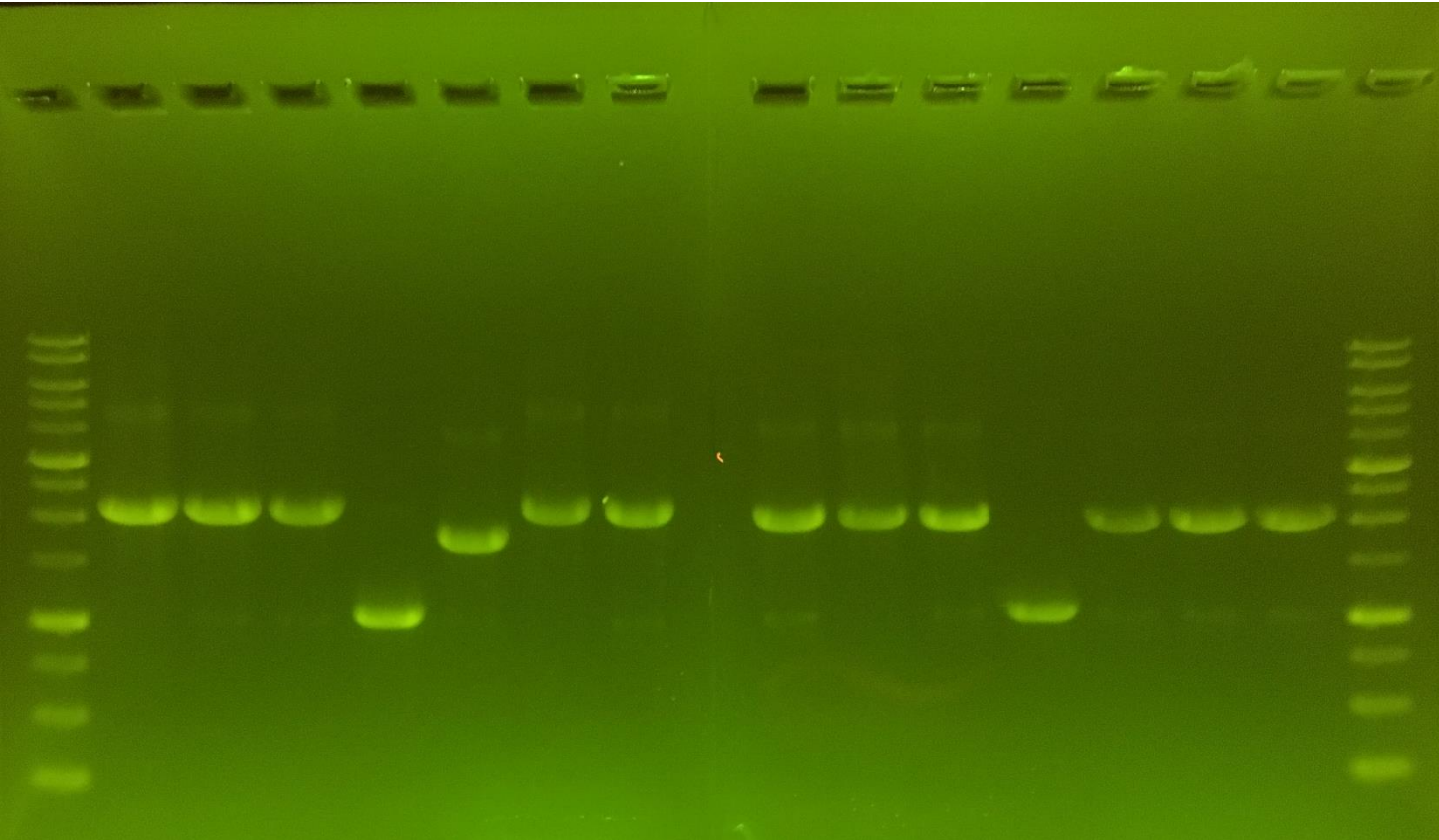
- Date: 2016.09.05
- Lane:
 1. XbaI-AOX1-PstI
 2. EcoRI-Pcons-RBS-SpeI

Ligation II: AOX1 + pSB1C3



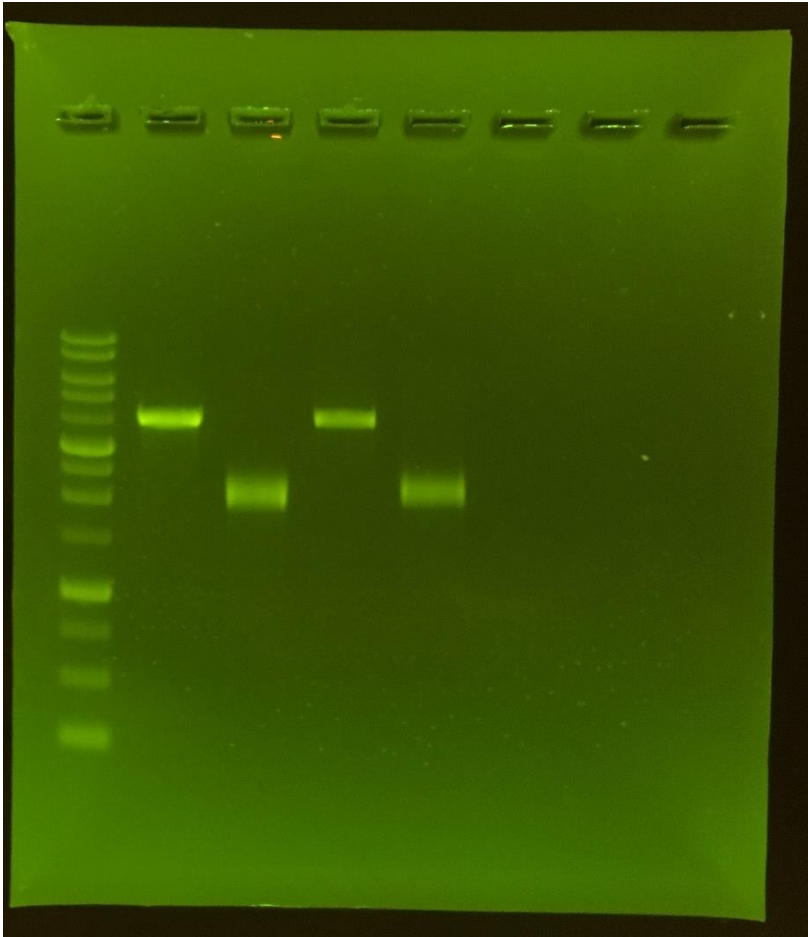
- Date: 2016.09.08
- Lane:
 1. pSB1C3: XbaI + PstI
 2. AOX1: XbaI + PstI

Colony PCR



- Date: 2016.09.09
- Clone: AOX1/pSB1C3
- Primer:
 1. VF2
 2. AOX1-PstI-R
- Lane: #1 ~ #14
- #1, 2 → Plasmid extraction → RE check

RE check



- Date: 2016.09.12
- Clone: AOX1/pSB1C3
- RE: EcoRI w/ or w/o PstI
- Lane:
 1. #1: EcoRI
 2. #1: EcoRI + PstI
 3. #2: EcoRI
 4. #2: EcoRI + PstI
- #1 → Sequencing

Sequencing

- Date: 2016.09.14
- Sequencing primers: VF2, VR
- Clone: AOX1/pSB1C3 #1
- File: “2016.09.14_AOX1_pSB1C3 (#1)”
- Result: **correct** → make a stock